

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Once amended) [A nucleic acid which comprises a polynucleotide] An isolated nucleic acid that encodes a fusion polypeptide, wherein the fusion polypeptide comprises:

a) a catalytic domain of a glycosyltransferase that catalyzes the transfer of a saccharide, from a saccharide donor comprising a nucleotide sugar, to an acceptor molecule; and

b) a catalytic domain of an accessory enzyme [which catalyzes a step in the formation of a nucleotide sugar which is a saccharide donor for the glycosyltransferase] that catalyzes the formation of the nucleotide sugar.

9. (Once amended) The nucleic acid of claim 1, wherein the accessory enzyme is selected from the group consisting of:

a GDP-mannose dehydratase[.];

a GDP-mannose 3,5-epimerase[, and];

a GDP-mannose 4-reductase;

a UDP-glucose 4' epimerase;

a UDP-GalNAc 4' epimerase;

a CMP-sialic acid synthetase;

a neuraminic acid aldolase;

an N-acetylglucosamine 2' epimerase;

a phosphate kinase selected from the group consisting of a pyruvate kinase, a myokinase, a creatine phosphate kinase, an acetyl phosphate kinase, and a polyphosphate kinase; and

a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase,

a GDP-mannose pyrophosphorylase, a GDP-fucose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase.

23. (Once amended) The nucleic acid of claim 1, wherein [the fusion polypeptide further comprises a linker peptide between the glycosyltransferase catalytic domain and the accessory enzyme catalytic domain] the catalytic domain of the glycosyltransferase and the catalytic domain of the accessory enzyme are joined by a peptide linker.

26. (Once amended) An expression vector which comprises [a] the nucleic acid of claim 1.

27. (Once amended) A host cell which comprises [a nucleic acid of claim 1] the expression vector of claim 26.

33. (Once amended) A method of producing a fusion polypeptide [that comprises:

- a) a catalytic domain of a glycosyltransferase; and
- b) a catalytic domain of an accessory enzyme which catalyzes a step in the formation of a nucleotide sugar which is a donor for the glycosyltransferase;

wherein the method comprises introducing a nucleic acid that encodes the fusion polypeptide into a host cell to produce a transformed host cell; and culturing the transformed host cell under conditions appropriate for expressing the fusion polypeptide], the method comprising:

- a) introducing into a host cell the expression vector of claim 26, under conditions where the host cell is transformed with the expression vector; and
- b) culturing the transformed host cell under conditions where the fusion polypeptide is expressed in the transformed host cell.

34. (Once amended) The method of claim 33[, wherein the fusion polypeptide is purified following its expression] further comprising a step of purifying the expressed fusion polypeptide.

35. (Once amended) The method of claim 33[, wherein the host cell is permeabilized following expression of the fusion polypeptide] further comprising a step of permeabilizing the host cell expressing the fusion polypeptide.

36. (New) The nucleic acid of claim 1, wherein the accessory enzyme is a pyrophosphorylase.